

CLAIMS

1. Pair of oligonucleotides, for use as a set in the amplification of a target sequence of the genome of SARS

5 Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 1: TACCTCTCCA GCTAGGATTT TCTACAGGTG TTAACCTAGT
AGCTGTACCG ACTGGTTATG TTGACACTGA AAATAACACA GAATTACCCA

10 GAGTTAATGC AAAACCTCCA CCAGGTGACC AGTTTAAACA TCTT,

SEQ ID 14: TCAGCCCCAG ATGGTACTTC TATTACCTAG GAACTGGCCC
AGAAGCTTCA CTT,

SEQ ID 23: TGCTCCAAGT GCCTCTGCAT TCTTGGAAT GTACGCATT
GGCATGGAAG TCACACCTT, or

15 SEQ ID 31: TGCCTATATG GAAGAGCCCT AATGTGTAAA ATTAATTTA
GTAGTGCTAT CCCCATGTGA TTTTAATAGC TT,

or the complementary sequence thereof,

a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

20 SEQ ID 2: ATGAATTACC AAGTCAATGG TTACCCCTAAT ATGTTTATCA
CCCGCGAAGA AGCTATTCGT CACGTTCGTG CGTGGATTGG CTTTGATGT,

SEQ ID 17: AGGTTTACCC AATAATACTG CGTCTTGGTT CACAGCTCTC
ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC

AATCAACACC AATAGTGGTC CAGATGACCA AAT,

25 SEQ ID 26: CCAAACGTGTC ACTAAGAAAAT CTGCTGCTGA GGCATCTAAA
AAGCCTCGCC AAAAACGTAC TGCCACAAAA CAGTACAACG TCACTCAAGC
ATTTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATT, or

SEQ ID 34: TACGATACAT AGTCTACTCT TGTGCAGAAT GAATTCTCGT
AACTAAACAG CACAAGTAGG TTTAGTTAAC TTTAATCTCA CATAGCAATC

30 TTTAATCAAT GT,

or the complementary sequence thereof.

2. Pair of oligonucleotides, according to claim 1,
consisting essentially of:

a first oligonucleotide comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 3: TCCACCAGGT GACCAGTTA AACATCTT,

5 SEQ ID 4: TAGTAGCTGT ACCGACTGGT TATGTT,

SEQ ID 5: TACCTCTCCA GCTAGGATT TCT,

SEQ ID 15: TCAGCCCCAG ATGGTACTTC T,

SEQ ID 16: TAGGAACCTGG CCCAGAAGCT TCACTT,

SEQ ID 24: TGCTCCAAGT GCCTCTGCAT TCTT,

10 SEQ ID 25: TTGGCATGGA AGTCACACCT T,

SEQ ID 32: TGCCTATATG GAAGAGCCC,

SEQ ID 33: TCCCCATGTG ATTAAATAG CTT,

or the complementary sequence thereof, and

a second oligonucleotide comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 6: ATGAATTACC AAGTCAATGG TTAC,

SEQ ID 7: GAAGCTATTC GTCACGTTCG,

SEQ ID 8: TGC GTGGATT GGCTTGATG T,

20 SEQ ID 18: AGGTTTACCC AATAATACTG CGT,

SEQ ID 19: AGATCCCTC GAGGCCAGGG CGT,

SEQ ID 20: ATAGTGGTCC AGATGACCAA AT,

SEQ ID 27: CCAAACGT ACTAAGAAAT CTGCT,

SEQ ID 28: CTCAAGCATT TGGGAGACGT GGT,

25 SEQ ID 29: CAGAACAAAC CCAAGGAAAT T,

SEQ ID 35: TACGATACAT AGTCTACTCT TGT,

SEQ ID 36: TAACTAAACA GCACAAGTAG GT,

SEQ ID 37: TAGCAATCTT TAATCAATGT,

or the complementary sequence thereof.

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3. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the replicase gene of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 1: TACCTCTCCA GCTAGGATT TCTACAGGTG TTAACCTAGT AGCTGTACCG
ACTGGTTATG TTGACACTGA AAATAACACA GAATTCACCA GAGTTAATGC

5 AAAACCTCCA CCAGGTGACC AGTTAAACA TCTT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 2: ATGAATTACC AAGTCAATGG TTACCCTAAT ATGTTTATCA

10 CCCGCGAAGA AGCTATTCTG CACGTTCTG CGTGGATTGG CTTTGATGT, or the complementary sequence thereof.

4. Pair of oligonucleotides, according to claim 3,
consisting essentially of:

15 a first oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

SEQ ID 3: TCCACCAGGT GACCAGTTA AACATCTT,

SEQ ID 4: TAGTAGCTGT ACCGACTGGT TATGTT,

20 SEQ ID 5: TACCTCTCCA GCTAGGATT TCT,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

25 SEQ ID 6: ATGAATTACC AAGTCAATGG TTAC,

SEQ ID 7: GAAGCTATTC GTCACGTTCG,

SEQ ID 8: TGCGTGGATT GGCTTGATG T,

or the complementary sequence thereof.

30 5. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the gene encoding the Nucleocapsid protein of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length
35 and comprising at least a fragment of 10 nucleotides of:

SEQ ID 14: TCAGCCCCAG ATGGTACTTC TATTACCTAG GAACTGGCCC
AGAAGCTTCA CTT, or the complementary sequence thereof, and
a second oligonucleotide being 10-50 nucleotides in length
and comprising at least a fragment of 10 nucleotides of:

5 SEQ ID 17: AGGTTTACCC AATAATACTG CGTCTTGGTT CACAGCTCTC
ACTCAGCATG GCAAGGAGGA ACTTAGATTC CCTCGAGGCC AGGGCGTTCC
AATCAACACC AATAGTGGTC CAGATGACCA AAT, or the complementary
sequence thereof.

10 6. Pair of oligonucleotides, according to claim 5,
consisting essentially of:

a first oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
of:

15 SEQ ID 15: TCAGCCCCAG ATGGTACTTC T,

SEQ ID 16: TAGGAAGTGG CCCAGAACCT TCACCTT,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
20 of:

SEQ ID 18: AGGTTTACCC AATAATACTG CGT,

SEQ ID 19: AGATTCCCTC GAGGCCAGGG CGT,

SEQ ID 20: ATAGTGGTCC AGATGACCAA AT,

or the complementary sequence thereof.

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7. Pair of oligonucleotides, for use as a set in the
amplification of a target sequence located within the gene
encoding the Nucleocapsid protein of the genome of SARS
Coronavirus, said pair consisting of:

30 a first oligonucleotide being 10-50 nucleotides in length
and comprising at least a fragment of 10 nucleotides of:

SEQ ID 23: TGCTCCAAGT GCCTCTGCAT TCTTGGAAT GTCACGCATT
GGCATGGAAG TCACACCTT, or the complementary sequence thereof,
and

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a second oligonucleotide being 10-50 nucleotides in length and comprising at least a fragment of 10 nucleotides of:

SEQ ID 26: CCAAACGTGTC ACTAAGAAAT CTGCTGCTGA GGCATCTAAA

5 AAGCCTCGCC AAAAACGTAC TGCCACAAAA CAGTACAACG TCACTCAAGC
ATTGGGAGA CGTGGTCCAG AACAAACCCA AGGAAATT, or the complementary sequence thereof.

8. Pair of oligonucleotides, according to claim 7,
10 consisting essentially of:

a first oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

SEQ ID 24: TGCTCCAA GTGCCTCTGC ATTCTT,

15 SEQ ID 25: TTGGCATGGA AGTCACACCT T,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of 10 nucleotides of a sequence selected from the group consisting of:

20 SEQ ID 27: CCAAACGTGTC ACTAAGAAAT CTGCT,

SEQ ID 28: CTCAAGCATT TGGGAGACGT GGT,

SEQ ID 29: CAGAACAAAC CCAAGGAAAT T,

or the complementary sequence thereof.

25 9. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the 3'-Non Coding Region (3'-NCR) of the genome of SARS Coronavirus, said pair consisting of:

a first oligonucleotide being 10-50 nucleotides in length
30 and comprising at least a fragment of 10 nucleotides of:

SEQ ID 31: TGCCTATATG GAAGAGCCCT AATGTGTAAA ATTAATTAA

GTAGTGCTAT CCCCATGTGA TTTAATAGC TT, or the complementary sequence thereof, and

a second oligonucleotide being 10-50 nucleotides in length
35 and comprising at least a fragment of 10 nucleotides of:

SEQ ID 34: TACGATACAT AGTCTACTCT TGTGCAGAAT GAATTCTCGT
AACTAACACAG CACAAGTAGG TTTAGTTAAC TTTAATCTCA CATAGCAATC
TTTAATCAAT GT, or the complementary sequence thereof.

5 10. Pair of oligonucleotides, according to claim 9,
consisting essentially of:

a first oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
of:

10 SEQ ID 32: TGCCTATATG GAAGAGCCC,

SEQ ID 33: TCCCCATGTG ATTTTAATAG CTT,

or the complementary sequence thereof, and

a second oligonucleotide comprising at least a fragment of
10 nucleotides of a sequence selected from the group consisting
of:

15 SEQ ID 35: TACGATACAT AGTCTACTCT TGT,

SEQ ID 36: TAACTAAACA GCACAAGTAG GT,

SEQ ID 37: TAGCAATCTT TAATCAATGT,

or the complementary sequence thereof.

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11. Pair of oligonucleotides, according to any of the
claims 1-10, wherein the first oligonucleotide is provided with
a promoter sequence recognized by a DNA dependent RNA
polymerase.

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12. Pair of oligonucleotides, according to claim 11,
wherein the first oligonucleotide consists essentially of the
sequence:

SEQ ID 9: aattctaata cgactcacta tagggAAGAT GTTTAACTG

30 GTCACCTGGT GGA,

SEQ ID 10: aattctaata cgactcacta tagggAACAT AACCAGTCGG
TACAGCTACT A,

SEQ ID 11: aattctaata cgactcacta tagggAGAAA ATCCTAGCTG
GAGAGGTA,

35 SEQ ID 39: aattctaata cgactcacta tagggAGAAG TACCATCTGG GGCTGA,

SEQ ID 40: *aattctaata cgactcacta tagggAAGTG AAGCTTCTGG*
 GCCAGTTCCCT A,
 SEQ ID 41: *aattctaata cgactcacta tagggAAGAA TGCAGAGGCA*
 CTTGGAGCA,
 5 SEQ ID 42: *aattctaata cgactcacta tagggAAGGT GTGACTTCCA TGCCAA,*
 SEQ ID 43: *aattctaata cgactcacta tagggGGGCT CTTCCATATA GGCA, or*
 SEQ ID 44: *aattctaata cgactcacta tagggAAGCT ATTAAAATCA*
 CATGGGA.

10 13. Pair of oligonucleotides, according to any of the
 claims 1-12, wherein each oligonucleotide being 15-30
 nucleotides in length and comprising at least a fragment of 18
 nucleotides, and preferably being 18-26 nucleotides in length
 and comprising at least a fragment of 20 nucleotides.

15 14. Oligonucleotide, for use as a probe to detect the
 amplified nucleic acid sequence resulting in the amplification
 of a target sequence located within the genome of SARS
 Coronavirus, said amplification being based on pair of
 20 oligonucleotides according to any of claims 1-13, said probe
 being 10-50 nucleotides in length and comprising at least a
 fragment of 10 nucleotides of:

SEQ ID 12: *GTTCGTGCCTT GGATTGGCTT TGATGTAGAG GGCTGTCATG*
CAACTAGAGA TGCTGT,
 25 SEQ ID 21: *GGCTACTACC GAAGAGCTAC CCGACGAGTT CGTGGTGGTG*
ACGGCAAAAT GAAAGAGCTC AGCCCCAGAT GGTACTTCTA TTACCTAGGA
ACTGGCCCAG AAGCTTCACT TCCCTACGGC GCTAACAAAG AAGGCATCGT
ATGGGTTGCA ACTGAGGGAG CCTTGAATAC ACCCAAAGAC CACATTGGCA
CCCGCAATCC TAATAACAAT GCTGCCACCG TGCTACAAC *TCCTCAAGGA*
 30 *ACAACATTGC CAAAAGGCTT CTACGCAGAG GGAAGCAGAG GCGGCAGTCA*
AGCCTCTTCT CGCTCCTCAT CACGTAGTCG CGGTAATTCA AGAAATTCAA
CTCCTGGCAG CAGTAGGGGA AATTCTCCTG CTCGAATGGC TAGCGGAGGT
GGTGAAACTG CCCTCGCGCT ATTGCTGCTA GACAGATTGA ACCAGCTTGA
GAGCAAAGTT TCTGGTAAAG GCCAACAAACA ACAAGGCCAA ACTGTCACTA
 35 *AGAAATCTGC TGCTGAGGCA TCTAAAAAGC CTCGCCAAA ACGTACTGCC*

ACAAAACAGT ACAACGTCAC TCAAGCATTT GGGAGACGTG GTCCAGAACAA
AACCCAAGGA AATTCGGGG ACCAAGACCT AATCAGACAA,
SEQ ID 38: GCCACCACAT TTTCATCGAG GC,
or the complementary sequence thereof, provided with a
5 detectable label.

15. Oligonucleotide, according to claim 14, wherein the probe is constituted by a molecular beacon, preferably consisting of:

10 SEQ ID 13: 5'-[6-FAM]-ccatgggCTGTCATGCAACTAGAGATGCTGTcccatgg-[DabSyl]-3',
SEQ ID 45: 5'-[6-FAM]-cgcgatGTTCGTGCCTGGATTGGCTTatcgcg-[DabCyl]-3',
SEQ ID 22: 5'-[6-FAM]-ccatgggCTACTACCGAAGAGCTACCCGACGAcccatgg-
15 [DabSyl]-3',
SEQ ID 30: 5'-[6-FAM]-ccatggACCAAGACCTAATCAGACAAccatgg-[DabSyl]-3',
SEQ ID 47: 5'-[6-FAM]-ccatgcGCCACCACATTTCATCGAgcatgg-[DabSyl]-3'.
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16. Use of an oligonucleotides' pair, according to any of the claims 1-13, in a nucleic acid amplification reaction or as a probe for the detection of SARS Coronavirus nucleic acid in a sample.

25 17. Method for the detection of SARS nucleic acid in a sample wherein the sample is subjected to a nucleic acid amplification reaction using a pair of oligonucleotides according to any of the claims 1-13 and suitable amplification reagents and the presence of any amplified nucleic acid is detected.
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18. Method according to claim 17, wherein the detection of any amplified nucleic acid is carried out by reacting the sample with an oligonucleotide according to claim 14 or 15
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under suitable hybridization conditions and detecting the presence of the label in any hybrids formed between the amplified sequence and the probe.

5 19. Method according to claim 17, wherein the amplification technique used is a transcription based amplification technique, preferably the NASBA, and the first oligonucleotide is provided with a promoter sequence recognized by a DNA dependent RNA polymerase.

10 20. Test kit for the detection of SARS Coronavirus in a sample comprising:

set of oligonucleotides according any of claims 1-13,
an oligonucleotide comprising a nucleic acid sequence
15 substantially complementary to at least part of the amplified nucleic acid sequence, provided with a detectable label,
according to claim 14 or 15, and
suitable amplification reagents.

20 21. Test kit according to claim 20, wherein suitable amplification reagents enable a transcription based amplification technique, preferably the NASBA.